REMARKS

Claims 26, 29, 30, 33, 34, 36 and 37 have been amended. Support for the amendments can be found throughout the specification including the Drawings and claims as filed originally. No new matter has been added. Applicant appreciates the Examiner's acknowledgement that claim 25 is allowed.

Applicant now turns to comments made by the Examiner in the Office Action as follows.

OFFICE ACTION

1. Claims 29-30, 33-34, and 36-37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

> The examiner states, "In claim 29, the phrase "said gene promoter" is indefinite because it is not clear if it is referring to the gene promoter of the previously set forth bone sialoprotein gene promoter or the previously set forth promoter of said matrix gla protein gene. Amendment of the claim to recite, for example, "of either or both of said gene promoters of said individual" will overcome this rejection. Claim 30 depends from claim 29 and is indefinite for the same reason. If applicant amends claim 29 as suggested, applicant it is suggested that applicant also amend claim 30 to recite "said amplified portion or portions."
>
> In claim 33, the phrase "said gene promoter" is indefinite because it is not clear if it is referring to the gene promoter of the previously set forth bone sialoprotein gene promoter or the previously set forth promoter of said osteopontin gene or the previously set forth matrix gla protein promoter. Amendment of the claim to recite, for example, "of one or more of said gene promoters of said individual" will overcome this rejection. Claim 34 depends from claim 33 and is indefinite for the same reason. If applicant amends claim 33 as suggested, applicant it is suggested that applicant also amend claim 34 to recite "said amplified portion or portions."
>
> In claim 36, the phrase "said gene promoter" is indefinite because it is not clear if it is referring to the gene promoter of the previously set forth bone sialoprotein gene promoter or the previously set forth promoter of said

> osteopontin gene or one of the other previously set forth gene promotesr. Amendment of the claim to recite, for example, "of one or more of said gene promoters of said individual" will overcome this rejection, Claim 37 depends from claim 36 and is indefinite for the same reason. If applicant amends claim 36 as suggested, applicant it is suggested that applicant also amend claim 37 to recite "said amplified portion or portions."

Applicant has amended the claims in accordance with the Examiner's suggestions. The rejection of claims 29-30, 33-34, and 36-37 on the basis of 35 U.S.C. 112, second paragraph should be obviated.

2. Claims 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, and 37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

> The Examiner states, "This rejection is written to address the portions of the rejoined claims that assert relationships between particular polymorphic alleles and phenotypes.
>
> Claim 26 recites the further assay of a polymorphism at position 242 of the matrix gla protein gene (SEQ ID NO: 26) and "associating the presence of said adenine in said sequence with a predisposition to a higher rate of loss of bone mass than when cytosine is present."
>
> Claim 27 recites the further assay of a polymorphisms at positions 1825 and 520of the osteopontin gene (SEQ ID NO: 27) and "associating the presence of said adenine in said sequence spanning base pair 520 with a predisposition to a higher rate of loss of bone mass than when guanine is present, and the presence of said thymine in the sequence spanning base pair 1825 with a predisposition to a lower bone mass than when cytosine is present."
>
> Claim 28 recites the further assay of a polymorphism at position 163 of the osteoprotegerin gene (SEQ ID NO: 28) and "associating the presence of said guanine in said sequence with a predisposition to a lower peak bone mass than when adenine is present."
>
> Additional claims 32 and 35 also recite these same further assays but represent different combinations with regard to which polymorphisms are actually assayed in combination with those recited in claim 25.
>
> Claim 25 is allowed. The remaining claims are either dependent from claim 25 or include all of the same limitations of claim 25 with regard to the bone

sialoprotein gene. The rejected claims, however, include these additional cited relationships between polymorphisms in additional genes and either peak bone mass or rate of loss of bone mass. The nature of the invention with regard to the rejected claims, thus, requires that the polymorphisms in the bone sialoprotein gene be associated with lower peak bone mass, but also require that the additional recited relationships be supported by the specification, as they are recited in the plain language in the claims. Thus, in this case, the independent claim 25 is enabled by the specification, but the claims which depend from this claim but recite additional relationships between polymorphisms and phenotypes are not enabled by the specification for the reasons set forth in this rejection.

Nature of the Invention

The Invention is concerned with providing a method for assessing an individual's predisposition to a lower peak bone mass via the genotyping of the promoter of the bone sialoprotein gene, and then further genotyping polymorphisms in different genes and associating these with either a predisposition to lower peak bone mass or rate of bone mass loss. Thus, the practice of the method relies on the showing of an association between a particular genotype and a particular calcification condition status.

Breadth of the claims

The claims are narrow with regard to the recited associations between particular polymorphic variants and associations.

Teachings in the Specification and Working Examples

The specification provides two novel polymorphisms within the 5' untranslated region of a gene taught by Kim *et al.* and referred to as the human bone sialoprotein promoter sequence, see GenBank L24756. Within this sequence, applicant identified polymorphisms at positions 1496 (A→G) and 1869 (G→A) wherein the first version is the version present in the published sequence and the second allele is the alternate allele identified by applicant (p. 7, lines 21-30). The specification refers to these variations as BSP-A1496G and BSP-G1869A, respectively.

The specification teaches an A→C polymorphism at position 242 of the matrix gla protein gene as set forth in SEQ ID NO: 26, The polymorphism is referred to in the specification as MGP-C242A.

The specification teaches an A→G polymorphism at position 520, and a C→T polymorphism at position 1825 of the human osteopontin gene as set forth in SEQ ID NO: 27. The polymorphisms are referred to in the specification as OPN-G520A and OPN-T 1825C.

The specification teaches a G→A polymorphism at position 163 of the osteoprotegerin gene as set forth in SEQ ID NO: 28.

In example 1 of the specification (beginning on page 22), applicant teaches the screening of the DNA from 133 women for the polymorphisms in the bone sialoprotien promoter sequence, in the matrix gla protein gene and in the osteopontin gene, via amplification of fragments of DNA and restriction

digestion. A comparison of allele frequencies versus measures of bone mineral content and bone density was made using statistical analysis, the results are given in Table 2, page 31. The example demonstrates that there is a significant association between the bone sialoprotein promoter sequence polymorphisms and bone mass as represented by bone mineral content and bone mineral density measurements (p. 31). Specifically, patients with the "A" allele at 1469 and/or the "G" allele at 1869 are more likely to have higher bone mass than patients with the opposite alleles (p. 31-32). Notably, within this table, however, there is no finding of a significant difference between bone mineral content (BMC) or bone mineral density (BMD) for the polymorphisms in the MGP or OPN genes, namely, for these two genes the statistical analysis resulted in the finding that the means are statistically the same between the two test groups using a t-test. Regarding these MGP and OPN, the specification states that these polymorphisms are not suitable for a prediction of BMC or BMD (p. 32), Referring to Figures 5 and 6, however, applicant asserts that the MGP-C242A and OPN-G520A may be associated with rate of bone loss, based on the shape of the curves presented in figures 5 and 6. However, no statistical analysis was preformed, and upon review of the figures, it is noteworthy that at each point on the graphs the standard deviations of the individual data points overlap, suggesting that the values at each point are not significantly different from one another. Further, for the MGP polymorphism, the % difference between the two genotypes over time gets smaller in the last time period, suggesting that perhaps the difference in the rate of bone loss may be decreasing. Thus, the figures do not provide a basis for drawing conclusions regarding the effect of the genotypes on rate of bone loss, since it is not clear from the data given if the trends observed are significant trends. Figure 8 shows a similar comparison for alleles of the OPN-T1825C polymorphism. Again, in this case the means appear to be so close to one another so as to not represent statistically different values. Thus, the figure does not support the assertion in the claims that this polymorphism is associated with bone mass.

The osteoprotegerin gene polymorphism is assayed in example 2, beginning on page 35. The specification teaches that the polymorphism is significantly associated with a difference in bone mineral content, and that when considered either of the two BSP polymorphisms is associated with both BMC and BMD. Thus, claim 28 is enabled insofar as it depends from claim 25, but insofar as it depends from claim 26 which recites a relationship between matrix gla protein promoter polymorphism and predisposition for higher rate of bone mass loss.

State of the prior art and Level of unpredictability

The prior art is silent with regard to the assertions set forth in the rejected claims.

However, there is a large body of knowledge in the prior art related to polymorphisms in general, and their association with diseases or disease states. The art is highly unpredictable with regard to the functionality of polymorphic sites in genomic DNA. After a screening assay identifies polymorphisms, it is

unpredictable whether any such polymorphisms (such as the two recited in the instant claims) would be associated with any phenotypic trait, such as a disease state or a physiological state. The instant specification demonstrates this unpredictability by demonstrating that the BSP polymorphisms are associated with peak bone mass but not with rate of bone loss. The prior art further exemplifies such unpredictability. For example, Hacker et al. were unable to confirm an association between a gene polymorphism and ulcerative colitis in a case where prior studies suggested such a relationship would exist since the relationship had been identified in a different population (Gut, 1997, Vol. 40, pages 623-627). Even in cases where an association between a particular gene and a disease state is known to exist, such as with the LPL gene and heart disease risk or the β-globin gene and sickle cell anemia, researchers have found that when using SNP (single nucleotide polymorphism analysis) it was difficult to associate SNPs with disease states or to even identify key genes as being associated with disease (Pennisi, Science, 281 (5384):1787-1789). Finally, in some cases where multiple polymorphisms are identified in a gene, some of these are demonstrated to be disease associated and some are not. Blumenfeld et al. (WO 99/52942) disclose a number of polymorphisms in the FLAP gene. While Blumenfeld et al. were able to demonstrate that some of these polymorphisms are associated with patients having asthma but some of these are not (see Figure 3). For example, the marker 10-35/390 was demonstrated to be associated with asthma, with a p value of 0.00229, while the marker 10-33/327 was determined to not have a statistical association with asthma (p=0.294). Thus, even for SNPs within the same gene, it is highly unpredictable as to whether a particular marker will be disease associated.

The level of skill in the pertinent art is quite high, i.e. generally a PhD in biochemistry, but the unpredictability in the art is higher. While the instant specification has disclosed that the two polymorphisms in the promoter of a human bone sialoprotein gene are associated with peak bone mass, the remaining relationships set forth in the claims are highly unpredictable. Thus, the claimed method directed towards the assessment of a predisposition to a selected calcification condition status requires the knowledge of unpredictable and potentially non-existent associations between the instantly disclosed polymorphisms and additional calcification condition statuses.

Quantity of Experimentation

The practice of the claimed invention commensurate in scope with the instant claims would require a high degree of experimentation to associate the disclosed polymorphisms with any or all calcification condition statuses. With respect to the disclosed polymorphisms within the bone sialoprotein gene, the practice of the claimed invention would require extensive further work to determine which calcification conditions can be predicted using even these polymorphisms. That this work would be unpredictable is exemplified in the specification which demonstrates that while the two disclosed polymorphisms in

> the bone sailoprotein gene may be predictors of bone mass within the tested population, they are not predictors of the rate of bone loss.
> **Conclusion**
> Thus, having considered each of these factors, namely the breadth of the claims, the high level of unpredictability in the related art, the lack of guidance in the specification and the prior art, and the high quantity of experimentation, it is concluded that it would require undue experimentation to practice the claimed invention commensurate in scope with the instant claims.".

Applicant respectfully disagrees. Regarding the rejections of Claims 26 to 30 and 32 to 37 as not being enabled, the Examiner has rejected Claim 26 on the ground that the specification does not sufficiently and convincingly demonstrate that the described MGP polymorphism is a valid predictor of the rate of bone loss. This conclusion is essentially based on the results shown in Figure 5. These are said by the Examiner to be of unclear statistical significance and to contain a hint that the difference between the two test groups is decreasing towards the end of the time period monitored.

Applicant submits that the Examiner has underrated the significance of the data presented. Just taking the 'convergence' point first, this is clearly of no relevance to the issue of enablement. It is unlikely that the two curves shown would ever meet, but even if they did, the allelic variation concerned would still reflect the propensity to a higher rate of bone loss during the period illustrated. That should be sufficient to satisfy the claim. Based on the claimed method, a physician would be able to tell a patient that their genetic make up indicated that statistically they had a greater/lesser chance (than the average age and sex matched person) of having had a lower peak bone mass and of having a higher/lower rate of loss of bone mass, at least during a broad range of years.

Secondly, overlap of standard deviation bars does not imply that there is no significant difference between the two groups. Many worthwhile biological tests do not provide a result that completely distinguishes between patient groups in the sense that an individual can be unambiguously allocated to one group or the other. It is sufficient for the purposes of the claim, and for a practically useful test, that a patient is established to have a higher probability of

belonging to one group rather than another. This is commonly all that can be achieved in a biological test having regard to the diversity between individuals. Thus, if the means of the two groups are separated but the range of individual values within the two groups overlaps, a valuable test result may be obtained.

One may think of commonly known examples. A test showing an 'elevated' level of blood cholesterol can be associated with an increased risk of heart disease but many individuals with a 'high' cholesterol level will never suffer from heart disease. Tests of that kind serve to provide information about risk factors (predispositions), not to provide a definitive diagnosis of disease, but are nonetheless of value.

If one looks at the results in Figure 5, the averages of the two groups are well separated at 12 years and remain so at 18 years. The standard deviation bars suggest that there will be some patients in the ZZ group who actually have a lower BMC at a given date than some patients in the Zz+zz group, but that is not the point. It is still possible to say that a patient determined to be ZZ has a lesser chance of or predisposition to having a relatively high rate of bone loss than a person determined to be Zz or zz.

The Examiner correctly notes that the teaching of the specification is that the mean values of the BMC and of the BMD for the two groups at the start are not statistically significantly different. It is noteworthy that the teaching of the specification is quite different regarding the differences in the means of the two groups at later times. Thus, as the Examiner has noted, the specification teaches in the Table on page 31 that there is no difference seen in BMC or BMD the 1977 data for MGP C242A. The teaching is quite different on page 32, lines 4 to 19 regarding the effect of following BMC over time in respect of the two OPN groups.

The Examiner has also rejected claim 27 relating to two polymorphisms of the OPN gene promoter and predisposition to higher rate of bone loss in respect of polymorphism OPN G520A

and to a lower bone mass in respect of OPN T1825C. As regards OPN G520A, the previously presented arguments (re MGP) apply.

The data in Figure 6, like that in Figure 5, shows that the averages of the two patient groups do diverge meaningfully over time and that allocation of a patient to one or the other group is a meaningful indicator of their predisposition to or chance of having a higher or lower rate of bone loss. Again, it is not necessary that the groups should be non overlapping for this to be useful.

As regards OPN T1825C, the position is quite different. Claim 27 of course requires that this should be measured in conjunction with the BSP polymorphism and Figure 9 clearly demonstrates the utility of this. As the Examiner remarks, Figure 8 reflects the comments in the specification that OPN T1825C taken alone is not an indicator of bone mass, but that is not what is covered by Claim 27. It is the data in Figure 9 that is relevant to the issue.

Claim 28 has been rejected only in so far as it depends on Claim 26, which was itself rejected. In this dependency, Claim 28 requires assessment of BSP (per Claim 25), MGP (per Claim 26) and OPG. There is, we understand, no dispute that the assessments of BSP and OPG in combination are useful and the objection depends entirely on the Examiner's arguments relating to the utility of the MGP determination. Applicant submits that these have been dealt with adequately above, so that this objection must fall way with the objection to Claim 26.

The Examiner's comments under the heading "State of the Prior Art and Level of Unpredictability" are in our submission not pertinent. The Examiner states that there is a large body of knowledge in the prior art related to polymorphisms in general and their association with disease or disease states and that the art is highly unpredictable with regard to the functionality of polymorphic sites in genomic DNA. The Examiner states that it is unpredictable whether any such polymorphisms would be associated with any phenotypic trait, such as a disease state or physiological state. It is not relevant whether such an association would be unpredictable in the

absence of data because the specification provides data. Instances in the prior art where workers had not been able to confirm an association with some particular disease state are equally irrelevant to the issue because such a relationship has been demonstrated in this case. Difficulties that workers have had in locating relevant sites of polymorphism within a gene known in general to have an association with a disease state equally are irrelevant as the present inventors have identified specific sites.

Toward the end of this section the Examiner states that "thus the claimed method directed towards the assessment of a predisposition to a selected calcification condition status requires the knowledge of unpredictable and potentially non-existent associations between the instantly disclosed polypmorphisms and additional calcification condition statuses".

With respect, this comment is not justified by the wording of the applicant's claims which are specific as to the particular calcification condition status in question. No question can arise of the skilled person needing to know anything of unpredictable or non-existent associations between the disclosed polymorphisms and any calcification condition status other than the particular one recited in the claims in respect of that polymorphism.

Equally, the Examiner's comments under the heading "Quantity of Experimentation" are not appropriate. The Examiner has stated that the practice of the claimed invention would require a high degree of experimentation to associate the disclosed polymorphisms with any or all calcification condition statuses. That is absolutely not required in order to practice what is claimed. The claims are specific as to the particular calcification condition status with which each disclosed polymorphism is to be associated. The Examiner goes on to suggest that the practice of the invention would require extensive further work even in connection with the BSP gene. That, of course, is contrary to the allowance of Claim 25 and equally is unjustified by the actual wording of the claims.

In view thereof, Applicant respectfully requests reconsideration and withdrawal of the rejection.

CONCLUSION

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. Applicant's representative would like to discuss this case with the Examiner to learn if any outstanding issues remain after consideration of this Amendment. If the Examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record. Although it is not believed that any further fee is needed to consider this submission, the Office is hereby authorized to charge our deposit account **04-1105** should such fee be deemed necessary.

Respectfully submitted,

Date: November 10, 2005

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